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Reduction of the occurrence of occult HBV infection in infants by increasing the dose of hepatitis B vaccine: a large prospective cohort study

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ABSTRACT

Occult hepatitis B virus (HBV) infection (OBI) has been observed among infants born to hepatitis B surface antigen (HBsAg)-positive mothers despite successful immunoprophylaxis. This study enrolled 549 infants [349 infants received a 10μg/dose of hepatitis B vaccine (HepB), and 200 infants received 20μg/dose HepB] born to HBsAg-positive mothers with HBV DNA load >6log$_{10}$IU/mL. The anti-HBs levels in the 10μg group were significantly lower than that in the 20μg group both at 7 [652.48 (564.05-754.82) *vs.* 1541.72 (1268.69-1873.51) mIU/mL, $P<0.001$] and 12 months old [257.44 (220.29-300.88) *vs.* 1073.41 (839.27-1372.78) mIU/mL, $P<0.001$]. The OBI incidence in the 10μg group was significantly higher than that in the 20μg group at both 7 [21.55% (25/116) *vs.* 7.56% (9/119), $P=0.002$] and 12 months old [17.07% (14/82) *vs.* 6.90% (6/87), $P=0.041$]. OBI incidence in infants with anti-HBs levels <100mIU/mL was higher than that of those with anti-HBs ≥100mIU/mL [35.71% (5/14) vs. 13.12% (29/221), $P=0.036$]. This study showed that increasing the immunisation dose from 10μg to 20μg significantly improved anti-HBs levels and decreased OBI incidence in infants with a high maternal viral load. We recommend 20μg HepB to treat this high-risk population.

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KEYWORDS Hepatitis B virus; hepatitis B vaccine; mother-to-child transmission; occult hepatitis B virus infection; post-vaccination serologic testing

Abbreviations

HBV	hepatitis B virus
HBsAg	hepatitis B surface antigen
HBeAg	hepatitis B e antigen
Anti-HBs	hepatitis B surface antibody
HepB	hepatitis B vaccine
OBI	occult hepatitis B virus infection
MTCT	mother-to-child transmission
HBIG	hepatitis B immunoglobulin
PVST	post-vaccination serologic testing
HCC	hepatocellular carcinoma.

Introduction

Chronic hepatitis B virus (HBV) infection is a major public health problem and affects 257 million individuals worldwide [1,2]. In 2018, there were 666,000 new cases of liver cancer globally, of which 360,000 were caused by HBV infection, accounting for 54.5% of all new cases [3]. In China, the universal vaccination of the hepatitis B vaccine (HepB) has aided in the prevention and control of HBV infection among children, with the hepatitis B surface antigen (HBsAg) carrying rate at 0.32% in children under 5 years old in 2014 [4]. In 2016, the World Health Assembly endorsed the Global Health Sector Strategy to eliminate viral hepatitis as a public health threat by reducing the incidence of chronic hepatitis B (CHB) to 10% and HBV-related mortality to 35% on the basis of the 2015 baseline, as well as the HBsAg prevalence to 0.1% among children under 5 years old by 2030 [5]. It is apparent that improved efforts are warranted to decrease the HBsAg prevalence among Chinese children under 5 years old to meet the 2030 prevalence goal.

Although the neonatal HepB vaccination is widely implemented and has been gradually improved, mother-to-child transmission (MTCT) still results in about 40%-50% of new HBV infections, especially in

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high prevalence areas [6]. Since the maternal hepatitis B e antigen (HBeAg) seropositivity and HBV DNA load are strongly correlated to the occurrence of immunoprophylaxis failure, 8%-30% of HBsAg-positive pregnant women with high viral loads may still transmit HBV to their children regardless of immunoprophylaxis [7]. Over the past decades, the prevalence of occult hepatitis B virus infection (OBI) and its potential clinical significance have been reported in the context of blood transfusion, liver transplantation, immunosuppressive conditions, and hepatocellular carcinoma (HCC) [8]. Recently, the occurrence of OBI in infants of HBsAg-positive mothers has been frequently reported despite immunoprophylaxis at birth, raising concerns that the current dose of HepB may not be completely effective in preventing infantile OBI by MTCT [9–18].

It has been reported that the percentage of OBI infants with low anti-HBs (HBsAb) (<100 mIU/mL) was significantly higher than that in non-infected infants, indicating the occurrence of OBI in infants may be due to the limited neutralising capacity provided by low anti-HBs titres [16]. A follow-up randomised controlled trial on healthy subjects demonstrated that the anti-HBs levels of the recipient could be enhanced by increasing the dose of HepB [19]. Currently, Chinese new-borns of HBsAg-positive mothers are recommended to receive 10μg HepB and one dose of hepatitis B immunoglobulin (HBIG; ≥100 IU) [4]. Several studies have shown that 20μg HepB has good immunogenicity and safety in new-borns, as well as long-term protective effects [20–23]. However, it remains unclear as to whether increasing the dose of HepB from 10μg to 20μg could significantly improve the immunoprophylaxis efficiency and reduce OBI incidence in infants born to HBsAg-positive mothers, especially for those born to HBeAg-positive and high HBV DNA load mothers. With a prospective cohort of infants born to HBsAg- and HBeAg-positive mothers with high-load HBV DNA (>6$\log_{10}$ IU/mL), the present study investigated the immune effect at 7 and 12 months old in children receiving 10μg or 20μg HepB combined with HBIG immunoprophylaxis regimes.

Material and methods

Subjects

In total, 549 infants born to HBsAg- and HBeAg-positive mothers with HBV DNA levels ≥6 $\log_{10}$-IU/mL were enrolled from the prospective mother-infant paired cohort as reported in our previous study [24]. All infants received three doses of recombinant yeast-derived HepB (10μg/0.5 mL or 20μg/1.0 mL; Dalian Hissen Biopharm Inc., Dalian, China or Shenzhen Kangtai Biological Products Co., Ltd., Shenzhen, China) at birth (within 12 h), 1, and 6 months, combined with one dose of HBIG (Hualan Biological Engineering Inc., Xinxiang, China) within 12 h of birth. Among them, 349 infants were vaccinated with 10μg/dose HepB combined with HBIG and 200 infants were vaccinated with 20μg/dose HepB combined with HBIG. All infants returned for post-vaccination serologic testing (PVST) at 7 months. At 12 months old, 296 infants in the 10μg group and 130 infants in the 20μg group were evaluated.

To analyse the occurrence of OBI at 7 months old, 116 and 119 anti-HBs-positive infants with enough serum sample to measure HBV DNA were selected out from the 10μg and 20μg group, respectively. Among them, 82 and 87 infants in the 10μg and 20μg group were followed up at 12 months old, respectively. The flowchart of enrolment and follow-up is shown in Figure 1.

Serological assays

Serum HBsAg, anti-HBs, and HBeAg levels were detected using an Abbott chemiluminescent microparticle immunoassay (Abbott Diagnostic, Chicago, IL, USA) on an Abbott i2000 system [25]. The detection range of the HBsAg assay was 0.05-250 IU/mL. Patients were considered anti-HBs positive if the level ≥10 mIU/mL. The results of the HBeAg assay were determined by the ratio of the sample relative light unit (RLU) to a cut-off RLU (S/CO) for each sample. Subjects with S/CO values higher than 1.0 were considered HBeAg-positive. HBV DNA load was quantitated by Abbott real-time HBV DNA assay (Abbott Molecular, IL, USA) using an Abbott m2000 system [25]. The lower limit of detection was 1.18 $\log_{10}$IU/mL (15 IU/mL, 51.2 copies/mL). Negative for HBV DNA was determined by assay results reported as "not detected" or "<1.18 $\log_{10}$IU/mL". HBV genotyping was performed using nested PCR as described previously [26].

Definitions

Immunoprophylaxis failure was defined as positive HBsAg and HBV DNA for infants at 7 months old. No response to immunisation was defined as both HBsAg and anti-HBs negative. Anti-HBs titres of 10-100, 100-1000, and ≥1000 mIU/mL were defined as low, medium, and high levels, respectively. OBI acquired from HBV MTCT was defined as negative HBsAg and positive HBV DNA.

Statistical analyses

Geometric mean concentration (GMC) and associated 95% confidence intervals (CIs) were calculated for anti-

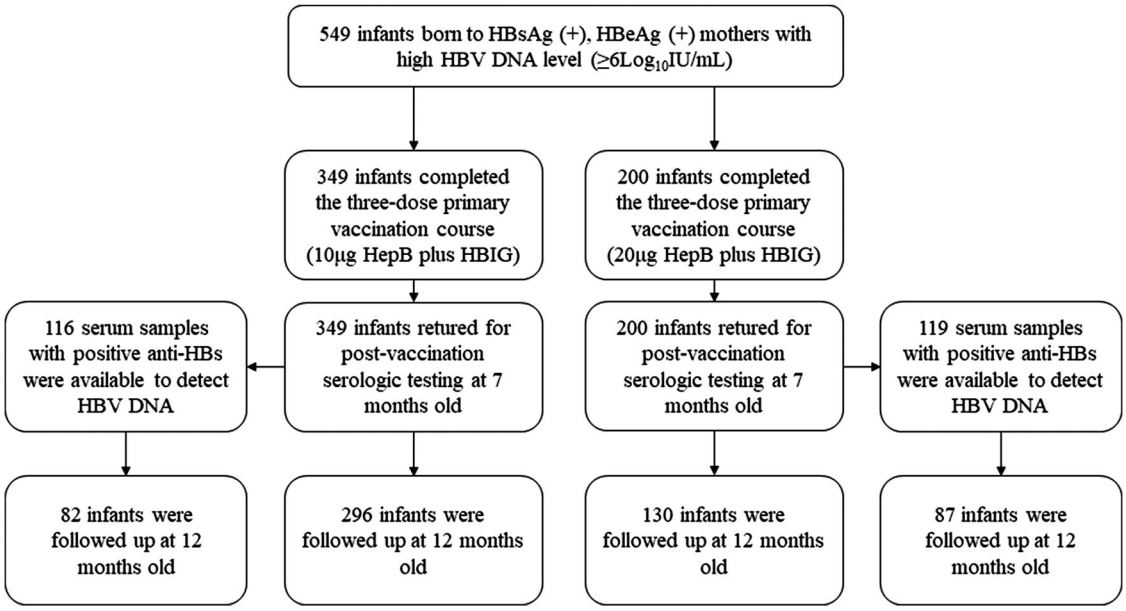

Figure 1. Flowchart of enrolment and follow-up.

HBs levels. Categorical variables were expressed as % (m/n) and examined by χ^2/Fisher's exact test. Non-normally distributed data were expressed as median and IQR or (range) and compared by Mann–Whitney U-test. All *P* values were two-tailed and a *P* value<0.05 was considered significant. Statistical analyses were performed using SPSS software V.25.0 (Chicago, IL, USA) and graphs were plotted using Graphpad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

Results

Baseline characteristics of infants

In this study, 549 infants born to HBsAg- and HBeAg-positive mothers with HBV DNA levels ≥ 6 $\log_{10}$IU/mL were enrolled. Among these infants, 349 and 200 infants received 10μg/dose and 20μg/dose HepB for three-dose vaccinations in combination with HBIG, respectively. As shown in Table 1, no significant differences in gender, birth weight, and feeding pattern were found between two groups. While more mothers underwent caesarean sections in the 20μg group than in the 10μg group, it has been shown that the risk of HBV transmission with breast feeding and delivery mode in infants who receive HepB and HBIG is negligible [24,27].

Immunoprophylaxis results of two immunisation doses

At 7 months old, 20 infants (5.73%, 20/349) who received 10μg HepB were diagnosed with immunoprophylaxis failure, with positive HBsAg [median: 4.58 (range: 2.19-5.24) $\log_{10}$IU/mL] and HBV DNA [8.39 (5.76-9.37) $\log_{10}$IU/mL], and 9 infants (4.50%, 9/200) in the 20μg group were found with positive HBsAg [4.70 (1.67-5.06) $\log_{10}$IU/mL] and HBV DNA [8.48 (6.34-9.13) $\log_{10}$IU/mL]. While a higher dose of HepB resulted in a lower incidence of immunoprophylaxis failure, this observation was not significant (4.50% *vs.* 5.73%, *P*=0.535). No significant differences in HBV DNA, HBsAg, HBeAg, and demographic characteristics of immunoprophylaxis failure infants between two groups were found (Table 2).

When considering the HepB response rate, all infants that demonstrated immunoprophylaxis failure were excluded. As shown in Supplementary Figure S1, 6 infants with anti-HBs ≤ 10 mIU/mL in the 10μg

Table 1. Baseline characteristics of infants under different immunization doses.

		10μg HepB	20μg HepB	*P*
Case number		349	200	
Gender	Male (%)	51.29% (179/349)	56.50% (113/200)	0.239
	Female (%)	48.71% (170/349)	43.50% (87/200)	
Birth weight (kg), average ± SD		3.41 ± 0.49	3.41 ± 0.41	0.85
Parturition manner	Vaginal (%)	47.85% (167/349)	19.00% (38/200)	**<0.001**
	Caesarean (%)	52.15% (182/349)	81.00% (162/200)	
Feeding pattern	Breast[a] (%)	33.24% (116/349)	29.00% (58/200)	0.304
	Artificial (%)	66.76% (233/349)	71.00% (142/200)	

[a]Breast-feeding included mixed feeding.
P values were calculated by student's *t*-test or χ^2 test.

Table 2. The virologic, serologic and demographic characteristics of infants with immunoprophylaxis failure under two immunization doses.

		10μg HepB + HBIG	20μg HepB + HBIG	*P*
Rate of immunoprophylaxis failure		5.73% (20/349)	4.50% (9/200)	0.535
Genotype	B (%)	20.00% (4/20)	0 (0/9)	0.149
	C (%)	80.00% (16/20)	88.89% (8/9)	
	B + C (%)	0 (0/20)	11.11% (1/9)	
HBsAg (log_{10}IU/mL), median (range)		4.58 (2.19-5.24)	4.70 (1.67-5.06)	0.908
HBeAg (S/CO), median (range)[a]		1206.35 (7.08-5843.92)	1287.17 (144.51-1587.79)	0.962
HBV DNA (log_{10}IU/mL), median(range)[b]		8.39 (5.76-9.37)	8.48 (6.34-9.13)	0.709
Birth weight (kg), average ± SD		3.43 ± 0.35	3.44 ± 0.54	0.944
Parturition manner	Vaginal (%)	60.00% (12/20)	22.22% (2/9)	0.109
	Caesarean (%)	40.00% (8/20)	77.78% (7/9)	
Feeding pattern	Breast (%)[c]	40.00% (8/20)	33.33% (3/9)	1
	Artificial (%)	60.00% (12/20)	66.67% (6/9)	

[a]an infant in 10μg HepB group was not available to measure HBeAg due to lack of serum sample.
[b]an infant in 20μg HepB group was not available to measure HBV DNA due to lack of serum sample.
[c]Breast-feeding included mixed feeding.
P values were calculated by student's *t*-test, Mann-Whitney U-test and Fisher's exact test.

group were diagnosed as having no response to HepB at 7 months old and were re-inoculated (3 doses of 10μg HepB under 0-1-6 procedure), 1 infant developed breakthrough infection (HBsAg: 5288.85 IU/mL, HBV DNA: 7.30 log_{10}IU/mL), and 5 infants generated anti-HBs successfully at 12 months old [157.44 (77.56-1101.87) mIU/mL]. In the 20μg group, all infants were responsive to HepB. The response rate of HepB was higher in infants who received 20μg HepB than those received 10μg HepB [100% (191/191) *vs.* 98.18% (323/329), *P*=0.090], however, no statistical significance was found.

In the 10μg group, 14.86% (48/323) of infants were not followed up at 12 months old. No significant difference in the GMC (95% CI) of anti-HBs at 7 months old was found between infants who were lost (n=48) and those successfully followed up (n=275) [720.11 (481.17-1077.71) *vs.* 641.34 (548.15-750.41) mIU/mL, *P*=0.557]. Among the 275 infants successfully followed up, the anti-HBs levels of 268 infants, including 3 infants who developed breakthrough infections, remained positive, and 7 infants, including 1 infant who developed breakthrough infection, turned negative at 12 months old. Overall, 1.78% (5/281) infants developed breakthrough infections at 12 months old, including 1 infant who was not responsive to HepB at 7 months old [HBsAg: 1.56 (0.25-5288.85) IU/mL, HBV DNA: 4.08 (2.78-7.30) log_{10}IU/mL]. In the 20μg group, 32.46% (62/191) of infants were not followed up at 12 months old, and no significant difference in the GMC (95%CI) of anti-HBs was found between infants who were lost (n=62) and those successfully followed up (n=129) [1570.79 (1129.28-2184.74) *vs.* 1527.94 (1197.02-1950.29) mIU/mL, (*P*=0.991)]. Among 129 infants who were successfully followed up, only 1 infant (0.78%, 1/129) developed breakthrough infection [HBsAg: 25.91 IU/mL, Anti-HBs: 74.78 mIU/mL, HBV DNA: 6.34 log_{10}IU/mL]. Notably, no infants' anti-HBs levels turned negative at 12 months old in the 20μg group.

Comparative analysis of anti-HBs levels under two immunisation doses

In total, 98.18% (323/329) and 100% (191/191) of infants who received 10μg and 20μg HepB successfully generated anti-HBs at 7 months old, respectively. At 12 months old, 275 and 129 infants in the 10μg and 20μg group were followed up, respectively. Based on three positive anti-HBs levels, specifically low level (10<anti-HBs<100mIU/mL), medium level (100≤anti-HBs<1000mIU/mL), and high level (anti-HBs≥1000mIU/mL), the ability of the two immunisation doses to stimulate the generation of anti-HBs was further analysed.

As shown in Figure 2A, the ratio of infants with low, medium, and high anti-HBs levels in the 10μg group was 8.36% (27/323), 53.87% (174/323), and 37.77% (122/323) at 7 months old, respectively. In the 20μg group, the ratio was 2.62% (5/191), 33.51% (64/191), and 63.87% (122/191), respectively. Therefore, more infants reached high anti-HBs level at 7 months old in the 20μg group than those in the 10μg group (63.87% *vs.* 37.77%, *P*<0.01). At 12 months old, the ratio of infants with negative, low, medium, and high anti-HBs levels in the 10μg group was 2.55% (7/275), 21.82% (60/275), 61.09% (168/275), and 14.55% (40/275), respectively. In the 20μg group, the ratio was 0, 7.75% (10/129), 34.88% (45/129), and 57.36% (74/129), respectively (Figure 2B). There were significantly more infants with high anti-HBs levels in the 20μg group than those in the 10μg group (57.36% *vs.* 14.55%, *P*<0.001). Taken together, more infants with high anti-HBs levels were found in the 20μg group at both 7 and 12 months old compared to infants in the 10μg group. When anti-HBs levels were separated at a higher resolution (0-10, 10-50, 50-100, 100-500, 500-1000, and ≥1000mIU/ml), the proportion of infants who received 20μg HepB was significantly lower than those who received 10μg HepB in both the 50–100 (0.5% *vs.* 4.0%, *P*<0.05) and 100–500 mIU/ml groups (16.2% *vs.* 29.4%, *P*<0.01), but

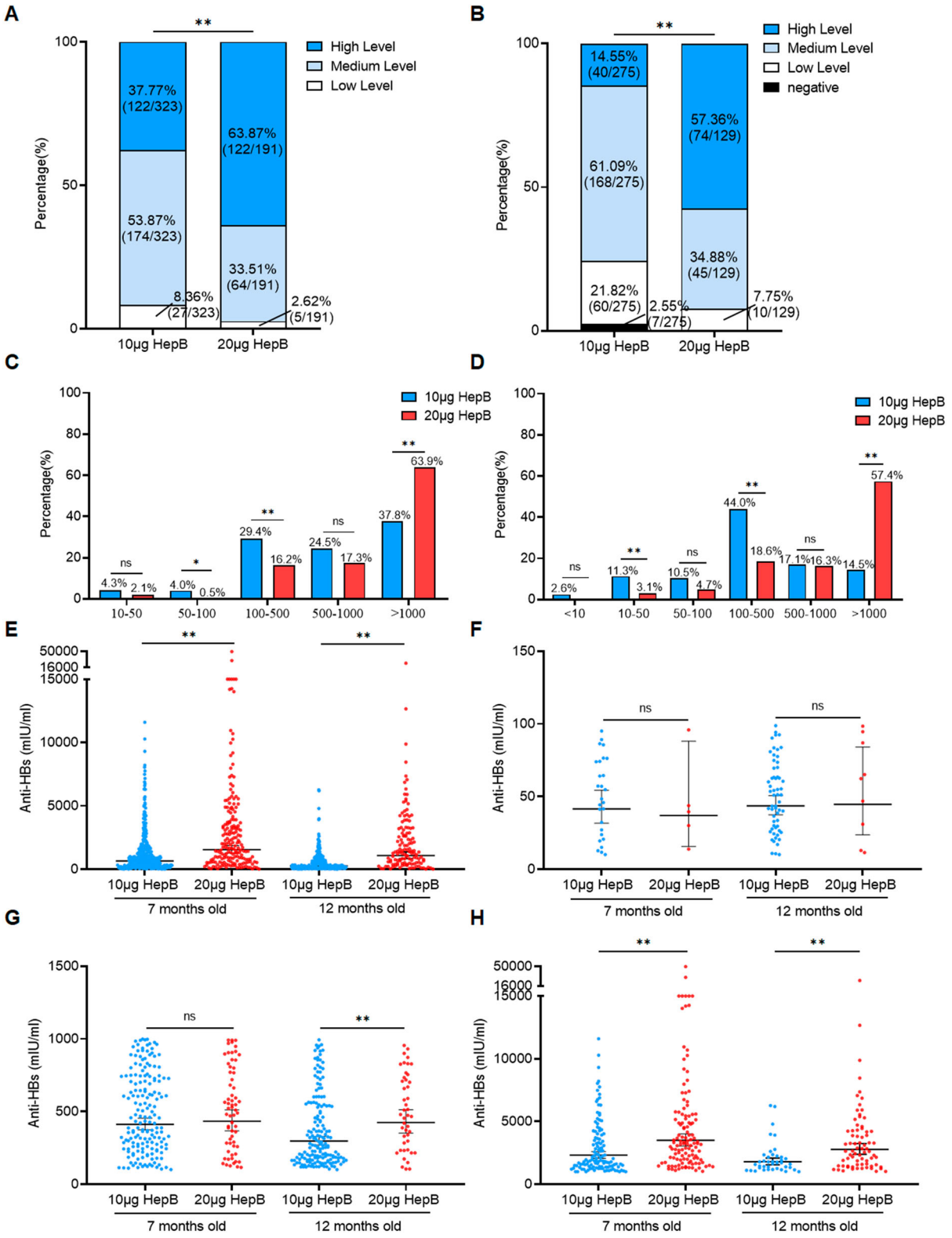

Figure 2. The distribution of infants at 7 and 12 months old with different levels of anti-HBs in the 10μg and 20μg groups. The distribution of infants with different levels of anti-HBs at 7 (A) and 12 (B) months old. The detailed distribution in two groups at 7 (C) and 12 (D) months old. (E) The total anti-HBs titer of infants in two groups at 7 and 12 months old. The anti-HBs titre of infants with low (F), medium (G), and high (H) anti-HBs level in two groups at 7 and 12 months old. *indicates $P<0.05$, **indicates $P<0.01$, "ns" indicates no significance, compared by Fisher's exact test or Mann-Whitney U-test.

significantly higher than those who received 10μg HepB in >1000 mIU/ml group at 7 months old (63.9% *vs.* 37.8%, $P<0.01$; Figure 2C). At 12 months old, there were more infants with a decreased anti-HBs level (from 500–1000 or >1000 mIU/ml to 100-500, 50-100, and 10-50mIU/ml) in the 10μg group than in the 20μg group.

Infants who developed breakthrough infection were ruled out when comparing anti-HBs titres. The total anti-HBs GMC (95%CI) was significantly higher in

infants who received 20μg HepB than those who received 10μg HepB [1541.72 (1268.69-1873.51) *vs.* 652.48 (564.05-754.82) mIU/mL, *P*<0.001] at 7 months old, and at 12 months old [1073.41 (839.27-1372.78) *vs.* 257.44 (220.29-300.88) mIU/mL, *P*<0.001] (Figure 2E). At low anti-HBs levels, no significant difference was found in anti-HBs GMC (95%CI) between the two groups at both 7 and 12 months old (Figure 2F). At medium anti-HBs levels, no significant difference was found in anti-HBs GMC (95%CI) between the two groups at 7 months old. However, when infants reached 12 months old, the anti-HBs GMC (95%CI) was significantly higher in infants who received 20μg HepB than those who received 10μg HepB [424.76 (352.29-512.15) mIU/mL *vs.* 300.13 (271.89-331.28) mIU/mL, *P*=0.002] (Figure 2G). At high anti-HBs levels, the anti-HBs GMC (95%CI) was significantly higher in infants who received 20μg HepB than those who received 10μg HepB at both 7 [3494.58 (3046.51-4008.56) *vs.* 2315.07 (2065.72-2593.85) mIU/mL, *P*<0.001] and 12 months old [2776.97 (2381.22-3238.17) *vs.* 1811.20 (1557.40-2106.69) mIU/mL, *P*<0.001] (Figure 2H). Taken together, compared to the 10μg group, significantly higher anti-HBs GMC in total and high anti-HBs levels were found in the 20μg group at both 7 and 12 months old, and in the 10μg group, anti-HBs GMC at medium anti-HBs levels decreased more rapidly than those in the 20μg group.

Furthermore, the kinetics of anti-HBs levels in each infant was analysed, excluding infants with breakthrough infection. As shown in Figure 3A and B, among infants who exhibited low anti-HBs levels at 7 months old, the anti-HBs levels of 3 infants (13.64%, 3/22) in the 10μg group turned negative at 12 months old, whereas all infants in the 20μg group remained positive. Among infants who exhibited medium anti-HBs levels at 7 months old, the anti-HBs levels of 28.77% (42/146) infants in the 10μg group decreased to lower than 100 mIU/mL at 12 months old, which was significantly higher than the number of infants in the 20μg group (12.20%, 5/41) (*P*=0.04). Interestingly, the anti-HBs levels of 2.05% (3/146) infants in the 10μg group dropped below the limit of detection (≤10 mIU/mL) at 12 months old, and the ratio of infants who rose to high anti-HBs levels was significantly lower in the 10μg group than in the 20μg group (2.05% *vs.* 31.70%, *P*<0.001) (Figure 3C and D). Among infants with high anti-HBs levels at 7 months old, the ratio of infants dropped to medium and low anti-HBs level at 12 months old were significantly higher in the 10μg group than in the 20μg group [57.28% (59/103) *vs.* 25.30% (21/83); 6.80% (7/103) *vs.* 1.21% (1/83), *P*<0.001], respectively. In addition, more infants sustained a high anti-HBs level in the 20μg group than those in the 10μg group (73.49% *vs.* 35.92%, *P*<0.001) (Figure 3E and F).

The occurrence of OBI in infants with positive anti-HBs

A total of 116 and 119 anti-HBs-positive infants whose serum samples were available to measure HBV DNA at 7 months old were analysed in the 10μg and 20μg group, respectively. As shown in Supplementary Table S1, no significant differences were found in the anti-HBs levels and demographic characters between the selected and total anti-HBs-positive infants, except that the ratio of the selected infants breast-fed was less than that of total anti-HBs-positive infants in the 10μg group (18.97% *vs.* 28.79%, *P*=0.039).

All infants who developed breakthrough infection (both HBV DNA and HBsAg were positive) were ruled out when calculating OBI incidence and relative HBV DNA levels. In the 10μg group, 21.55% (25/116) of infants were positive for HBV DNA [2.01 (1.20-3.71) $\log_{10}$IU/ml] and diagnosed as OBI at 7 months old, and the OBI incidence was significantly higher than that in the 20μg group (7.56%, 9/119) (*P*=0.002) (Figure 4A). As shown in Table 3, the anti-HBs levels of OBI infants in the 10μg group was significantly lower than that in the 20μg group [587.68 (325.84-1058.28) *vs.* 1688.36 (388.96-7302.98) mIU/mL, *P*=0.040]. Additionally, HBV DNA level was also higher in the 10μg group than in the 20μg group [2.01 (1.20-3.71) *vs.* 1.42 (1.19-2.46) $\log_{10}$IU/mL, *P*=0.066], yet this was not significant. At 12 months old, no significant difference in the drop-out rate between the 10μg and 20μg group [29.31% (34/116) *vs.* 26.89% (32/119), *P*=0.680] was found. The OBI incidence in the 10μg group was significantly higher than that in the 20μg group [17.07% (14/82) *vs.* 6.90% (6/87), *P*=0.041]. Moreover, the HBV DNA level was higher in the 10μg group than that in the 20μg group [1.86 (1.25-3.36) *vs.* 1.24 (1.20-3.14) $\log_{10}$IU/mL, *P*=0.051], yet there was no statistical significance.

To analyse the relationship between OBI incidence and anti-HBs levels, all infants were divided into two groups based on their anti-HBs levels at 7 months old. As shown in Figure 4B, 35.71% (5/14) infants with anti-HBs <100mIU/mL were diagnosed as OBI, which was significantly higher than that of infants with anti-HBs ≥ 100 mIU/mL (13.12%, 29/221) (*P*=0.036). Moreover, 4 out of 5 OBI infants with anti-HBs <100mIU/mL were found in the 10μg group.

The flowchart of the results of HBV DNA detection for the two immunisation doses is shown in Figure 5. For infants with negative HBV DNA at 7 months old, 30 infants in each group were lost to follow-up at 12 months old. HBV DNA was positive for 16.39% (10/61) and 8.75% (7/80) of infants at 12 months old in the 10μg and 20μg groups, respectively. For infants with positive HBV DNA at 7 months old, 4 and 2

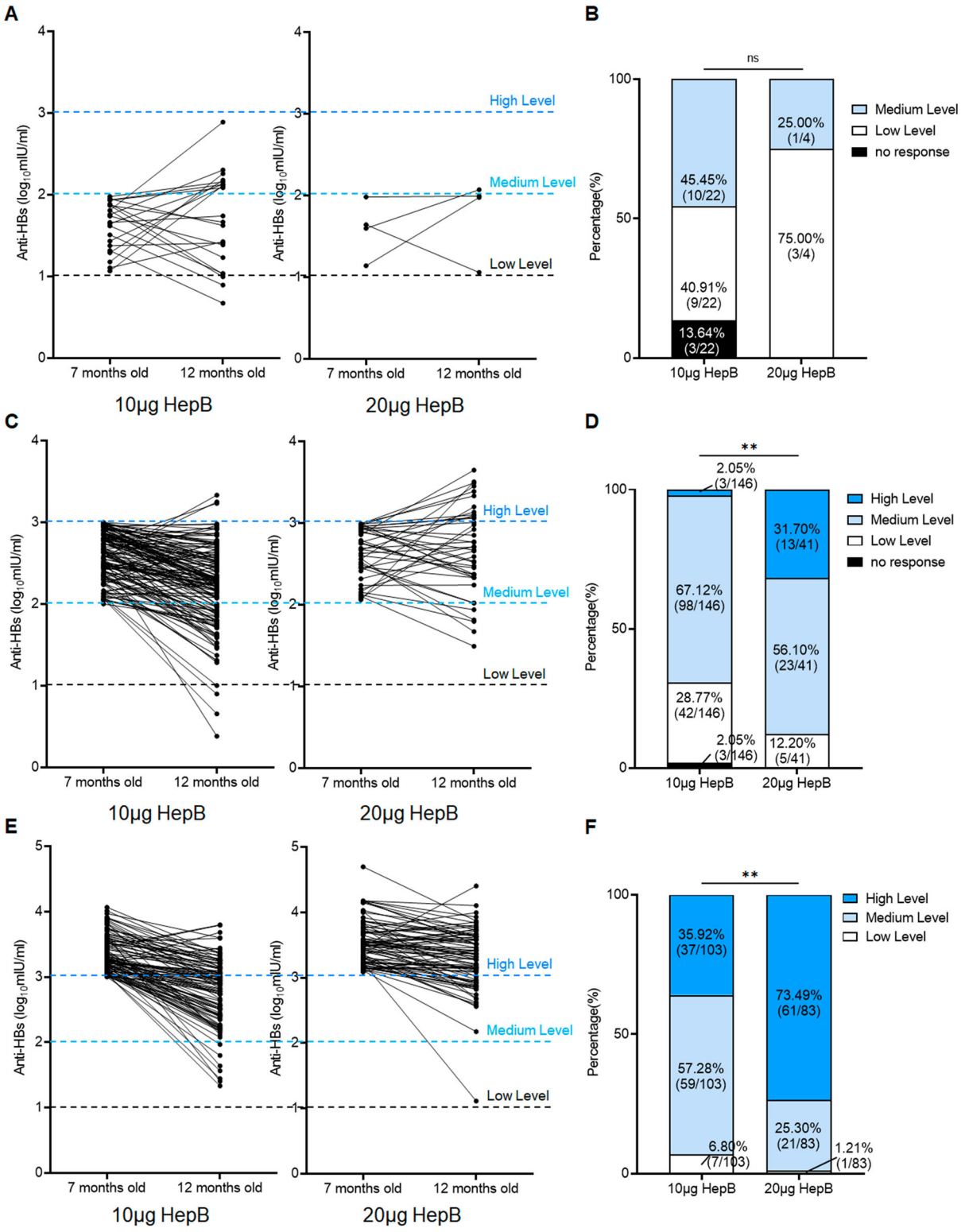

Figure 3. The dynamic changes of anti-HBs levels in infants with low, medium and high anti-HBs level at 7 months old. The dynamic changes of anti-HBs levels in infants with low anti-HBs level at 7 months old in two groups (A), and the distribution of their anti-HBs levels at 12 months old (B). The dynamic changes of anti-HBs levels in infants with medium HBsAg level at 7 months old in two groups (C), and the distribution of their anti-HBs levels at 12 months old (D). The dynamic changes of anti-HBs levels in infants with high HBsAg level at 7 months old in two groups (E), and the distribution of their anti-HBs levels at 12 months old (F). **indicates $P<0.01$, "ns" indicates no significance, compared by Fisher's exact test.

infants in the 10μg and 20μg HepB group were lost to follow-up at 12 months old, respectively. HBV DNA turned negative for 76.19% (16/21) and 100% (7/7) infants at 12 months old in the 10μg and 20μg HepB groups, respectively.

Discussion

The immunoprophylaxis failure and occurrence of OBI among infants born to HBsAg- and HBeAg-positive mothers with HBV DNA ≥ 6 $\log_{10}$IU/mL appears to

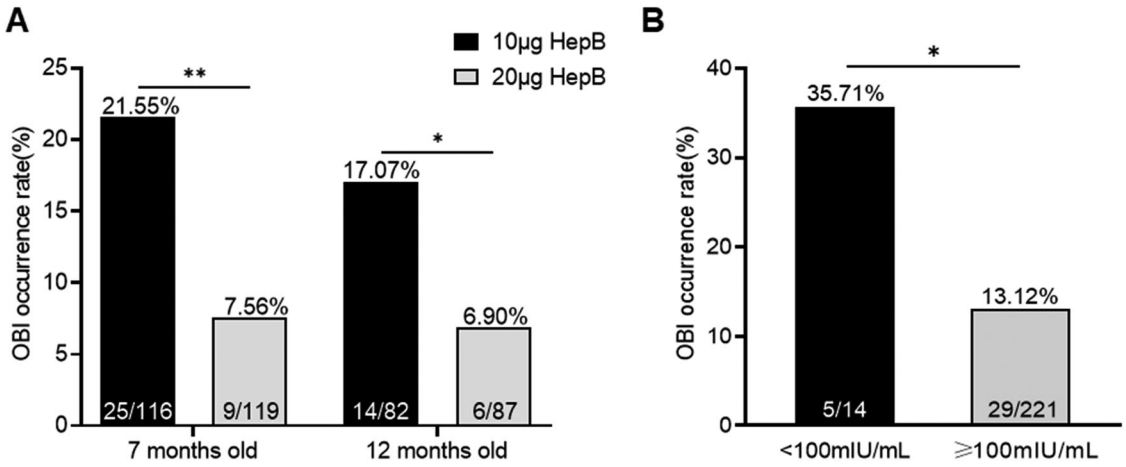

Figure 4. The OBI incidence of infants in the 10μg and 20μg groups. (A) The OBI incidences of infants at 7 and 12 months old in two groups. (B) The OBI incidences in infants with anti-HBs<100mIU/mL and infants with anti-HBs≥100mIU/mL at 7 months old. * indicates $P<0.05$, ** indicates $P<0.01$, compared by χ^2 test.

be a major challenge in controlling HBV infection and achieving the goal of eliminating viral hepatitis. This study aimed to explore whether increasing the vaccine dose to infants born to mothers with HBeAg-positive and high HBV DNA levels (>6 $\log_{10}$IU/mL) could improve the efficacy of immunoprophylaxis. No mothers enrolled in this study received any antiviral treatment during their pregnancy, making this cohort suitable to investigate the efficacy of different vaccination strategies.

The present study found that at 12 months old, more infants in the 10 μg group exhibited vaccine failure or developed breakthrough infection than those in the 20 μg group, even though the difference was not significant, which might be due to the small number of subjects enrolled in this study. Moreover, compared to the 10μg HepB group, the infants' response to the vaccine in the 20μg group showed encouraging results, whereby 20μg HepB could stimulate infants to generate significantly higher anti-HBs levels than 10μg of treatment. More importantly, there were less infants in the 20μg group who developed breakthrough infection, and no infants' anti-HBs levels turned negative at 12 months old, compared to those who received 10μg HepB. When anti-HBs levels were separated, the anti-HBs GMC and ratio of infants with high anti-HBs levels (≥1000 mIU/mL) in the 20μg group were all significantly higher than that in the 10μg group at both 7 and 12 months old. In addition, the ratio of infants with low anti-HBs levels (anti-HBs<100 mIU/mL) in the 20μg group was significantly lower than that in the 10μg group at both 7 and 12 months old. In addition, anti-HBs levels decreased in more infants in the 10μg group and were maintained at a higher level in infants in the 20μg group. Moreover, the proportion of infants with anti-HBs levels that rose from medium to high in the 20μg group was significantly higher than that in the 10μg group, which might be due to the higher the dose of HepB and the stronger the immune memory. Accordingly, compared to the 10μg group, the stronger immune memory in the 20μg group might cause the increase in anti-HBs levels and protect infants from breakthrough infection when exposed to HBV. Our results demonstrated that 20μg HepB could stimulate a stronger immune response, generate higher anti-HBs at 7 months old, and

Table 3. The virologic, serologic and demographic characteristics of OBI infants under two immunization doses.

		10μg HepB	20μg HepB	*P*
OBI number at 7 months		25	9	
HBV DNA (Log_{10}IU/mL), median (range)		2.01 (1.20-3.71)	1.42 (1.19-2.46)	0.07
GMC (95%CI) at 7 months		587.68 (325.84-1058.28)	1688.36 (388.96-7302.98)	**0.04**
Maternal HBV DNA (Log_{10}IU/mL), median (range)		8.29 (6.57-8.90)	8.34 (6.49-8.58)	0.76
Maternal HBsAg (Log_{10}IU/mL), median (range)		4.55 (3.21-4.83)	4.47 (4.27-4.67)	0.56
Maternal HBeAg (Log_{10}S/CO), median (range)		3.15 (2.87-3.34)	3.16 (2.96-3.25)	0.67
Birth Weight (kg), average ± SD		3.49 ± 0.51	3.19 ± 0.25	0.10
Delivery mode	Vaginal (%)	40.00% (10/25)	11.11% (1/9)	0.21
	Caesarean (%)	60.00% (15/25)	88.89% (8/9)	
Feeding pattern	Breast (%)[a]	12.00% (3/25)	11.11% (1/9)	1.00
	Artificial (%)	88.00% (22/25)	88.89% (8/9)	
OBI number at 12 months		14	6	
HBV DNA (Log_{10}IU/mL), median (range)		1.86 (1.25-3.36)	1.24 (1.20-3.14)	0.05
GMC (95%CI) at 12 months		326.81 (136.05-785.05)	732.82 (179.68-2988.82)	0.28

[a]Breast-feeding included mixed feeding.
P values were calculated by student's *t*-test, Mann-Whitney U-test and Fisher's exact test.

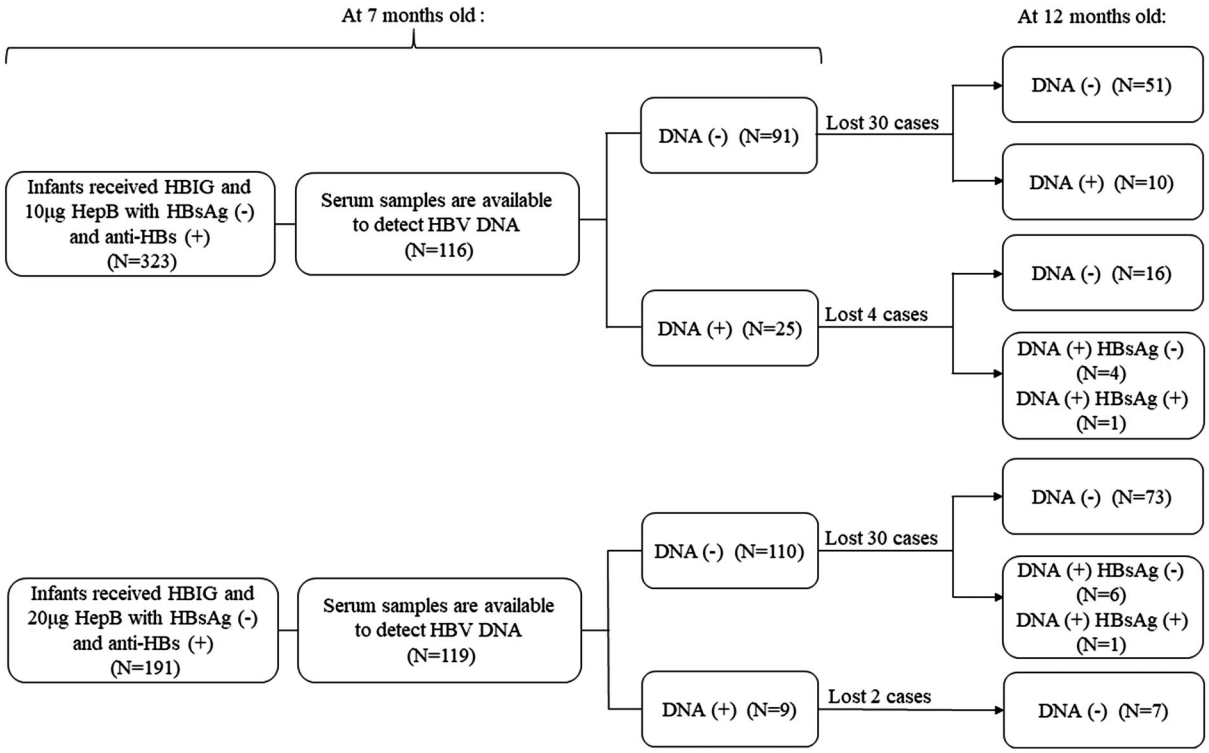

Figure 5. Flowchart of the results of HBV DNA detection in the 10μg and 20μg groups.

maintain a relatively higher level at 12 months old, when compared to infants treated with 10μg HepB.

OBI is currently defined as the presence of replication-competent HBV DNA in the liver and/or the blood of people who test negative for HBsAg with currently available assays [8]. The donor with OBI can transmit HBV to the recipient through blood transfusion or liver transplantation, and the recipient may develop typical hepatitis B [28]. Individuals with OBI can experience HBV reactivation when they receive cancer chemotherapy or other immunosuppressive therapies, and may promote the progression of chronic liver disease to cirrhosis, and even HCC based on several oncogenic mechanisms of OBI, including production of pro-oncogenic proteins and the propensity of the viral DNA to integrate into the host's genome [8,29–31]. In brief, OBI is a type of chronic HBV infection that needs to be controlled by immunoprophylaxis to achieve the goal of eliminating viral hepatitis by 2030.

The incidence of OBI among infants born to HBsAg-positive mothers reportedly ranges from 1.6%-66% [9–18]. The discrepancy in OBI incidence might be due to the different HBV prevalence rates, maternal HBV DNA load and HBeAg status, experience of maternal antiviral therapy, immunisation procedures, age of infants, sample size, sensitivity and specificity of the adopted detection methods, etc. In this study, we found that the OBI incidence for infants at 7 months old was significantly higher in the 10μg group than that in the 20μg group, which might be due to the lower anti-HBs levels in the 10μg group. Furthermore, we found that the incidence of OBI in infants with anti-HBs levels <100 mIU/mL was significantly higher than in infants with anti-HBs levels ≥100 mIU/mL, which was consistent with the blood donors with OBI [32,33]. Moreover, in this prospective study, all the infants with sufficient serum sample collected were measured for HBV DNA at 12 months old irrespective of whether they were diagnosed as OBI or not at 7 months old. Interestingly, 76.19% (16/21) OBI infants in the 10μg group and all (7/7) OBI infants in the 20μg group turned HBV DNA negative at 12 months old, and 16.39% (10/61) non-OBI infants in the 10μg group and 7.50% (6/80) non-OBI infants in the 20μg group developed OBI at 12 months old. The anti-HBs levels of 16 infants (10 in the 10μg group and 6 in the 20μg group) who developed OBI at 12 months old were lower than those infants whose HBV DNA remained negative at both 7 [959.48 (522.88-1760.76) *vs.* 1205.09 (938.64-1547.04) mIU/mL, P=0.428] and 12 months old [327.48 (145.71-736.04) *vs.* 619.91 (467.95-821.30) mIU/mL, P=0.106], even though no statistical significance was observed. Furthermore, nested PCR for S gene (nt 253 - nt 756) amplification was performed for the available blood samples of 7 pairs of mothers and 12-month-old infants. As shown in Supplementary Figure S2, HBV DNA sequences between mothers and their paired infants were clustered together, indicating that secondary maternal infections might occur. As all sequences clustered into several subgroups, cross contamination could be excluded.

Although antiviral intervention can significantly reduce the risk of immunoprophylaxis failure [34],

the safety of antiviral drugs for both mothers and infants remains controversial [35]. As close person-to-person contact (probably by open cuts and sores) is one of the main transmission routes of HBV, infants with low initial anti-HBs levels are likely at a high risk of HBV breakthrough infection from close contact with their mothers. Therefore, we suggest HepB at 20μg/dose for infants born to mothers who are HBeAg-positive and exhibit a high HBV DNA load (>6 $\log_{10}$IU/mL). This large, prospective cohort study demonstrated that a high dose of HepB reduces the incidence of OBI, which provides evidence for improving current immunisation procedures in this high-risk population.

Declarations

Ethics approval and consent to participate: The cohort data involved in the study was approved by the Bioethics Committees of Peking University. We confirm that we have all necessary consents from any individuals involved in the study.

Availability of data and materials: The datasets used during the current study are available from the corresponding author on reasonable request.

Author contributions: Jie Li and Jie Wang designed the study. Yi Li, Zhixiu Liu, Yarong Song, Yiwei Xiao, Lili Li, Feng Ding, Jie Wang and Jie Li performed the study. Jing Jiang, Xiangjun Zhai, Jianxun Liu, Liguo Zhu, Jie Jiang and Jie Li were in charge of patients enrolled in this study. Yi Li, Zhixiu Liu, Yarong Song, Yiwei Xiao, Lili Li, Zhongping Duan, Jia Liu, Hui Zhuang, Huaibin Zou, Jie Wang and Jie Li analyzed data. Yi Li and Zhixiu Liu drafted the manuscript. Jie Li and Jie Wang contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript. Jie Li and Jie Wang are the study guarantors.

Disclosure statement

No potential conflict of interest was reported by the author (s).

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